ORIGINAL ARTICLE

8-oxo-dG Levels in Gingival Crevicular Fluid and Increased Micronuclei Number as Markers of Panoramic Radiation Exposure

Rurie Ratna Shantiningsih, Rellyca Sola Gracea, Silviana Farrah Diba, Ryna Dwi Yanuaryska

Department of Dentomaxillofacial Radiology, Faculty of Dentistry, Universitas Gadjah Mada, Jalan Denta No. 1 Sekip Utara, 55281, Sleman, Yogyakarta, Indonesia

ABSTRACT

Introduction: Micronuclear formation is possibly related to the increased levels of 8-oxo-dG, which is a DNA damage. Micronuclei is used as a biomarker of genotoxic damage due to panoramic radiographic exposure. The 8-oxo-dG levels detected in gingival crevicular fluid (GCF). This study aimed to determine the correlation of 8-oxo-dG levels in GCF with an increase in the number of micronuclei as the parameter of the effects of panoramic radiographic exposure. **Methods:** Randomized sampling was applied to 10 patients for each digital and conventional technique, indicated as the panoramic radiographic exposure at the radiology installation of RSGM UGM Prof. Soedomo. Smears on the gingival mucosa of each subject were obtained before exposure and 10 days after exposure, then stained via the Feulgen–Rossenbeck-modified method. Samples of GCF was acquired before and shortly after exposure to measure 8-oxo-dG levels by enzyme-linked immunosorbent assay (ELISA). Data were analyzed statistically with paired t-test and Pearson correlation test. **Results:**The 8-oxo-dG levels increased after the panoramic radiographic exposure in conventional and digital techniques. However, paired t-test showed no significant difference ($p > 0.05$). In addition, the 8-oxo-dG levels were significantly correlated ($p < 0.01$) with the increased number of micronuclei ($r = 0.749$). The correlation of digital techniques ($r = 0.879$) was higher than that of analog techniques ($r = 0.673$). **Conclusion:** The 8-oxo-dG levels in GCF increased because of panoramic radiographic exposure and correlated with the increased number of micronuclei in the gingival mucosa, as shown in conventional and digital radiographs.

Keywords: Micronuclei, Gingival mucosa, 8-oxo-dG levels, GCF, Panoramic radiography

Corresponding Author:
Rurie Ratna Shantiningsih, PhD
Email: rurieratna@ugm.ac.id
Tel: +61 813 2871 2276

INTRODUCTION

Panoramic radiography is one of the supporting examination techniques to establish a definite diagnosis in dentistry. The panoramic radiography provides a wider range image covering the maxilla and mandible in one appearance. Numerous theories about the effects of radiation state that panoramic ionizing radiation can cause cell damage as a result of oxidative reactions (1). Riberio (2) reported that exposure to panoramic radiography elicits effects in the form of micronuclear formation associated with the mutagenic effects of X-rays. This observation is consistent with the theory that a small dose of radiation administered in dentistry still poses some risks (3).

The number of micronuclei is often examined and used as a biomarker of genotoxic damage in various epidemiological studies. Their use is non-invasive and provides accurate results because definite parameters are utilized to detect micronuclei and distinguish them from other damaged cells (4). Micronuclear formation indicates genomic instability and increasing risk of cancer (5). Micronuclei are an additional form of the nucleus that appears during microscopic examinations; they measure about one-fifth to one-third of the size of the main nucleus and have the same colour as the main nucleus (6).

Shantiningsih (7) confirmed a significant increase in the number of micronuclei after the human gingival mucosa is exposed to panoramic radiography. This result supported the findings of Cerquiera (6), who showed that panoramic exposure increases the number of micronuclei in a patient's gingival mucosa. In previous studies, the number of micronuclei and the expression of 8-oxo-dG increase after panoramic exposure, as detected in the gingival mucosa of rabbit through immunohistochemical (IHC) techniques. This increase in the number of micronuclei is also followed by an increase in the expression of 8-oxo-dG (8). The DNA damage caused by oxidative reactions results in the release of a marker called 8-oxo-dG, as induced by

panoramic exposure (9). Furthermore, the expression of 8-oxo-dG can be measured using samples from the gingival crevicular fluid (GCF) via enzyme-linked immunosorbent assay (ELISA), so invasive actions are not required (10). The detection of 8-oxo-dG expression by ELISA can be used as a simple technique instead the micronuclei counting to monitor the effect of radiation.

The effect of X-rays from panoramic radiography is stochastic, and no definite dose limit value is set to cause an effect (3). In terms of the effective dose used, previous studies found that the effects of exposure to conventional and digital panoramic radiography are not significantly different (2). However, other studies have reported a significant difference in the number of micronuclei exposed to digital and conventional panoramic radiography at the radiology installation at RSGM UGM Prof. Soedomo (10).

According to the radiation protection principle of as low as reasonably achievable, even small doses of radiation exposure affect an exposed tissue (11). Our study aimed to determine the correlation between 8-oxo-dG levels in GCF and the increase in the number of micronuclei, which would be used as a parameter to analyse the effects of exposure to panoramic radiography.

MATERIALS AND METHODS

Study design and sample

This study received an Ethical Clearance from the Research Ethics Committee of the Faculty of Dentistry, Universitas Gadjah Mada (001428/KKEP/FKG-UGM/EC/2018) to meet the research ethics requirements. All participants included in this research gave written informed consent to join this research. A total of 10 participants were taken digital panoramic radiography and 10 participants taken conventional panoramic radiography for diagnosis and treatment in Prof. Soedomo UGM Dental Hospital, Indonesia as the subjects in this study. They were required to fulfil the following inclusion criteria: (1) Have systemic healthy condition; (2) Age between 18 and 30 years; (3) Were not taken radiographic exposure for at least two weeks prior to the study; and (4) Agree to be a subject by signing an informed consent and the exclusion criteria: (1) The presence of systemic diseases; (2). Periodontal tissue inflammation at anterior teeth. The subject characteristics can be seen in the Table I.

Sample collection

Subjects were asked to rinse their mouth before they smeared the sample on their mucosa. Sampling was carried out by swabbing the gingival mucosa with a cervical brush in accordance with the methods described by Shantiningsih and Diba (10). The sample cells obtained from gingival mucosa which taken in one smeared were given 2 drops of 0.09% NaCl solution before the number of micronuclei was analysed.

Table I: Subject Characteristics

Characteristics		Conventional n (%)	Digital n (%)
Sex			
	Female	100	100
Age			
	18-20	10	10
	21-23	50	30
	24-27	40	60
Bracket wearing		20	30
Alcohol consumption		0	0

Subsequent smears were taken 10 days after panoramic radiographic exposure.

In all the same subjects, GCF samples were taken from the gingival sulcus of the anterior teeth by using paper points before and after panoramic radiography exposure (Figure 1). Before the GCF was collected, the teeth to be addressed were isolated using a cotton roll. The GCF was obtained with a paper point inserted in the gingival sulcus gap next to the buccal and left for 1 min. If the sample was contaminated with blood, sampling was repeated, but the sample was replaced with the tooth next to it. The GCF samples at the paper point were placed in a 500 µL Eppendorf tube and stored at –20 °C until the sample size was met.

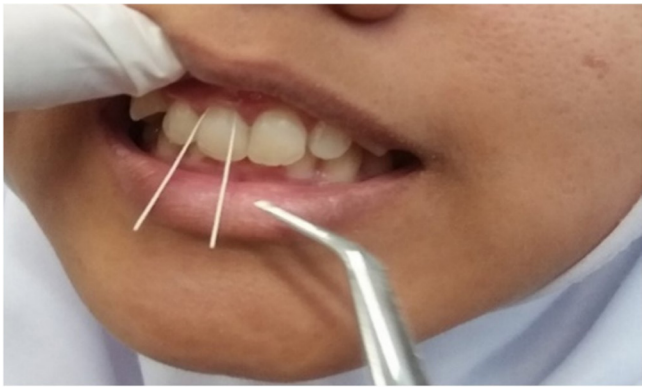

Figure 1: Procedure of GCF Collection. GCF collected from the labial gingival sulcular of the anterior teeth

Micronuclei analysis

Micronuclear staining was conducted to analyse the number of micronuclei in gingival cells of 100 cells scored per sample's slide. First, the sample was fixed on a slide by using absolute alcohol at –20 °C and placed in a staining jar containing 1 N HCl at 56 °C for 10 min. Second, the sample was stained with the Feulgen–Rossenbeck-modified method by immersing it in Schiff's reagent solution and countering it with fast green. The micronuclei were counted by observing the cells under a 40Ч magnification microscope. Then, the cell that appeared to have an additional nucleus called the micronuclei was added using a hand tally counter. The micronuclei was around the main core, providing the same staining results as the main core and the

smaller one, whose diameter was about 1/3 that of the main core. Afterward, the number of micronuclei was analysed (Figure 2).

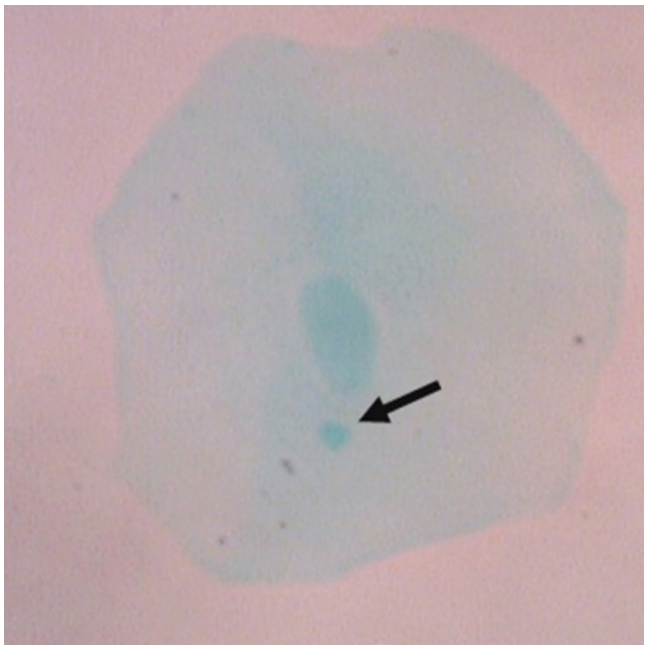

Figure 2: Micronuclei Image. Image of a micronuclei (black arrow) stained with Schiff's reagent and counterstained with fast green (light microscopy at 40x) detected in gingival cell after panoramic radiography exposure

ELISA

The GCF samples at the paper point in the Eppendorf tube were removed from the deep freezer at −20 °C and placed at room temperature. Then, 50 µL of PBS was added and centrifuged at 2500 rpm and 4 °C for 15 min. All the samples were placed in 50 µL microtiter plate wells and given 8-oxo-dG antibodies. The plate was covered with foil and incubated at room temperature for 10 min. Afterward, 50 µL of anti-8-OhdG antibodies was added to each well and incubated at room temperature for 1 hour. Subsequently, 100 µL of secondary antibody–enzyme conjugate was added to all wells and incubated at room temperature for 1 hour. Then, 100 µL of the substrate solution was added to each well and incubated at room temperature between 2 and 30 min. The enzymatic reaction was stopped by adding 100 µL of the stop solution to each well. The absorbance of each microwell was observed at the main wavelength of 450 nm by using a spectrophotometer.

Statistical data analysis

Paired t-test analysis was conducted to examine the differences in 8-oxo-dG levels before and after conventional and digital panoramic radiographies. Independent t-test analysis was performed to compare the increased levels of 8-oxo-dG between conventional and digital techniques. The correlation between the levels of 8-oxo-dG and the number of micronuclei during digital and conventional techniques was examined with a Pearson correlation test.

RESULTS

Figure 3 shows the 8-oxo-dG levels before and after exposure to conventional and digital panoramic radiographies. In particular, the 8-oxo-dG levels before and after exposure of the conventional panoramic groups were higher than those of the digital. The 8-oxo-dG levels after exposure to digital panoramic radiography showed no statistical significance ($p = 0.211$) compared with those after exposure to conventional panoramic radiography ($p = 0.018$). The difference in the 8-oxo-dG levels before and after exposure was assessed in the form of increasing levels. In particular, the degree of increase in 8-oxo-dG levels in the conventional radiography group was higher than that in the digital radiography group (Figure 4), but the difference was not significant ($p > 0.05$).

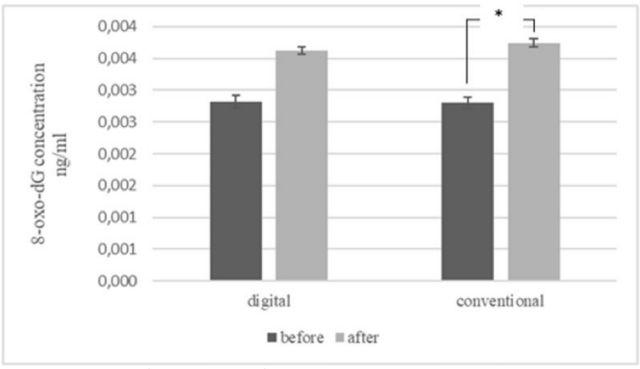

Figure 3: Level of 8-oxo-dG. Differences in 8-oxo-dG levels in the groups before and after exposure to conventional and digital panoramic radiographies shows the statistically significant ($p<0.05$)

Figure 4 shows the increase in the number of micronuclei in the groups exposed to conventional and digital panoramic radiography. The independent t-test revealed a significant difference between conventional and digital panoramic radiography ($p = 0.01$). Figure 5 illustrate the levels of 8-oxo-dG and the number of micronuclei upon exposure to digital and conventional radiographies, respectively. The result of Pearson correlation test shown in the Table II indicated that the levels of 8-oxo-dG significantly correlated with the number of micronuclei in the groups exposed to digital and conventional panoramic radiography ($p < 0.05$).

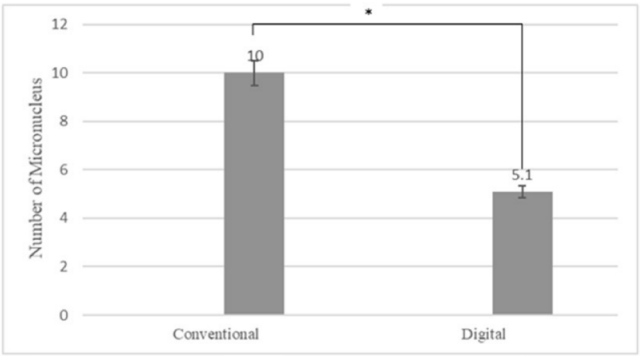

Figure 4: Increased number of micronuclei. There is a siginicant difference in the degree of increase in the number of micronuclei from total number in a group between the groups exposed to conventional and digital panoramic radiographies

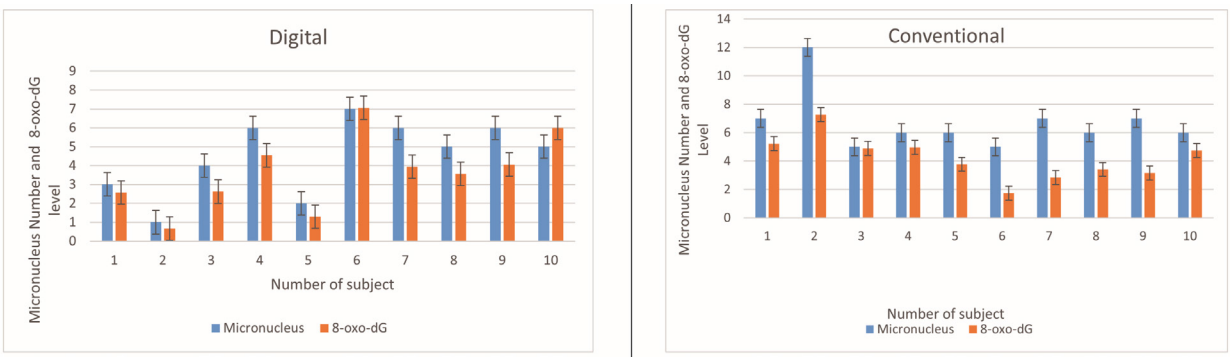

Figure 5: Data distribution of the levels of 8-oxo-dG and the number of micronuclei in digital and conventional panoramic radiography. This figure strengthens Table II to describe the significantly correlation of 8-oxo-dG level to the micronuclei number in each group ($p < 0.05$).

Table II: The Result of correlation test between the levels of 8-oxo-dG and the number of micronuclei

Type of Radiography	N	Pearson correlation	Sig
Digital	10	0.879	0.001*
Conventional	10	0.673	0.033*

*Significancy P<0.05

DISCUSSION

The micronuclei were identified in accordance with the following criteria: 1) round/oval with a smooth border, 2) size ranging from one-fifth and one-third of the size of the main nucleus, 3) located near but separated from the main nucleus, 4) Feulgen positive, 5) staining similar to the main nucleus that showed chromatin distribution, and 6) not overlapping with other cells. Only the cells with the full cytoplasm were counted as shown in Figure 2 (5-7).

In this study, the levels of 8-oxo-dG were different before and after exposure to conventional and digital radiography (Figure 3). However, their differences were not significant in the group exposed to digital panoramic radiography. The levels of 8-oxo-dG were higher in conventional panoramic radiography than those in digital panoramic radiography, but the increase in these levels was not significantly different (Figure 4). This result confirmed that the effects of exposure to conventional and digital panoramic radiographies can increase the 8-oxo-dG levels although the dose of exposure to digital panoramic radiation was lower than that of conventional exposure (3).

Figure 4 shows the difference in the degree of increase in the number of micronuclei between conventional and digital panoramic radiographies. Their number increased because panoramic radiography exposure triggered an oxidative reaction (8). An increase in the number of micronuclei exposed to digital panoramic radiography was lower than that exposed to conventional panoramic radiography. These findings were consistent with previous results, which showed that the number of micronuclei in the gingival mucosa exposed to digital panoramic radiography significantly differs from that of micronuclei in the gingival mucosa exposed to conventional panoramic radiography at the radiology installation of RSGM Prof. Soedomo (10). This observation is related to the theory explaining that digital panoramic radiography can reduce the dose of exposure compared with conventional panoramic radiography. Thus, the use of digital panoramic radiography is considered one of the efforts of radiation protection (12). The number of micronuclei mostly increases in the buccal mucosa rather than in the gingival mucosa because the former is the first area exposed to X-rays during panoramic radiography (13). This observation is possibly related to the speed of turnover between the buccal mucosa and the gingiva, indicating that the buccal mucosa can regenerate faster than the gingiva mucosa (14).

The levels of 8-oxo-dG correlated with the increased number of micronuclei (Figures 5). Pearson correlation analysis also revealed that the number of micronuclei significantly correlated with the levels of 8-oxo-dG ($p < 0.05$). These findings were consistent with previous results, which showed a strong correlation between the increased number of micronuclei and the levels of 8-oxo-dG (8). In a previous study, 8-oxo-dG levels were obtained through immunohistochemistry (IHC) of samples of gingival mucosal slices from rabbits. In this study, the 8-oxo-dG levels in GCF were analysed with ELISA to develop an easy and non-invasive technique. Current and previous studies showed that 8-oxo-dG can be detected in GCF with ELISA (10). GCF is a material collected from the periodontal sulcus, which has diagnostic value in non-invasive measures (15).

Low-dose ionizing radiation remains a potential inducer of inflammatory reactions and can be detected using 8-oxo-dG markers. The production of 8-Oxo-dG is associated with DNA damage caused by oxidative stress (16). Extracellular 8-oxo-dG is considered a sensitive biomarker of individual responses to oxidative stress and can be measured through ELISA (17). Intraoral extracellular 8-oxo-dG can be detected using saliva and GCF, which provide 8-oxo-dG contents equivalent to blood levels detected via ELISA (18). This observation

corroborates our findings, which showed an increase in the 8-oxo-dG levels in GCF because of the exposure to digital and conventional panoramic radiographies through ELISA. This increase in the 8-oxo-dG levels strongly correlated with the increase in the number of micronuclei.

Oxidative stress due to ionization is related to nutritional status, especially the presence of antioxidants (16). Nutrition deficiencies cause an increased risk of DNA damage due to oxidative stress. Therefore, additional antioxidants are needed for protection against radiation.

CONCLUSION

8-Oxo-dG levels in GCF correlates with the increased number of micronuclei. Therefore, 8-oxo-dG can be used as a parameter of detecting the effect of radiographic exposure. This study had a limitation because of the number of subjects was limited. By the results we strongly suggested that radiation protection should be considered in the patient undergoing panoramic radiographic exposure. However, further analyses are required to investigate antioxidant agent for radiation protection to minimize the radiation effects.

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